

Claims

What is claimed is:

5

1. A method for producing a hydrolysate from a proteinaceous substrate which comprises subjecting the substrate to a polypeptide having aminopeptidase activity and an endopeptidase, wherein the polypeptide having aminopeptidase activity is selected from the group consisting of:

10

(a) a polypeptide having an amino acid sequence which has at least 95% identity with amino acids 17 to 771 of SEQ ID NO:2;

15

(b) a polypeptide which is encoded by a nucleic acid sequence which hybridizes under high stringency conditions with (i) nucleotides 49 to 2396 of SEQ ID NO. 1, or (ii) the cDNA sequence corresponding to nucleotides 49 to 2396 of SEQ ID NO. 1, wherein high stringency conditions are defined as prehybridization and hybridization at 42°C in 5X SSPE, 0.3% SDS, 200 µg/ml sheared and denatured salmon sperm DNA, and 50% formamide, and washing with 2X SSC, 0.2% SDS at 65°C; and

(c) a fragment of (a) or (b), wherein the fragment has dipeptidyl aminopeptidase activity.

20

2. The method of claim 1, wherein the polypeptide having aminopeptidase activity comprises an amino acid sequence which has at least 95% identity with amino acids 17 to 771 of SEQ ID NO:2.

25

3. The method of claim 2, wherein the polypeptide having dipeptidyl aminopeptidase activity comprises an amino acid sequence which has at least 97% identity with amino acids 17 to 771 of SEQ ID NO:2.

30

4. The method of claim 1, wherein the polypeptide having dipeptidyl aminopeptidase activity comprises the amino acid sequence of SEQ ID NO:2.

5. The method of claim 1, wherein the polypeptide having dipeptidyl aminopeptidase activity consists of the amino acid sequence of SEQ ID NO:2 or a fragment thereof having dipeptidyl aminopeptidase activity.

35

6. The method of claim 5, wherein the polypeptide having dipeptidyl aminopeptidase

activity consists of the amino acid sequence of SEQ ID NO:2.

7. The method of claim 6, wherein the polypeptide having dipeptidyl aminopeptidase activity consists of amino acids 17 to 771 of SEQ ID NO:2.

5

8. The method of claim 2, wherein the polypeptide having dipeptidyl aminopeptidase activity is obtained from an *Aspergillus* strain.

10 9. The method of claim 8, wherein the polypeptide having dipeptidyl aminopeptidase activity is obtained from an *Aspergillus oryzae* strain.

15 10. The method of claim 1, wherein the polypeptide having dipeptidyl aminopeptidase activity is encoded by a nucleic acid sequence which hybridizes under high stringency conditions with (i) nucleotides 49 to 2396 of SEQ ID NO. 1, or (ii) the cDNA sequence corresponding to nucleotides 49 to 2396 of SEQ ID NO. 1, wherein high stringency conditions are defined as prehybridization and hybridization at 42°C in 5X SSPE, 0.3% SDS, 200 µg/ml sheared and denatured salmon sperm DNA, and 50% formamide, and washing with 2X SSC, 0.2% SDS at 65°C.

20 11. The method of claim 10, wherein the polypeptide having dipeptidyl aminopeptidase activity is obtained from an *Aspergillus* strain.

12. The method of claim 11, wherein the polypeptide having dipeptidyl aminopeptidase activity is obtained from an *Aspergillus oryzae* strain.

25

13. The method of claim 1, wherein the polypeptide having dipeptidyl aminopeptidase activity is encoded by the nucleic acid sequence contained in plasmid pMWR52 which is contained in *E. coli* NRRL B-21682.

30 14. The method of claim 1, wherein the polypeptide having dipeptidyl aminopeptidase activity acts synergistically with an aminopeptidase to hydrolyze a polypeptide.

15. The method of claim 1, wherein the protein hydrolysate is enriched in Ala, Arg, Asp, Gly, and/or Val.

5 16. A protein hydrolysate produced by the method of claim 1.

17. A food product comprising the protein hydrolysate of claim 16.

18. A method for producing a hydrolysate from a proteinaceous substrate which comprises
10 subjecting the substrate to a polypeptide having aminopeptidase activity and an endopeptidase, wherein the polypeptide having aminopeptidase activity has the following physicochemical properties: (a) a pH optimum at about pH 8.7 determined after incubation for 5 minutes at ambient temperature in the presence of 2.9 mM Ala-Pro-para-nitroanilide; (b) a temperature stability of 90% or more, relative to initial activity, after incubation for 20
15 minutes at 65°C, pH 7.5 in the absence of substrate, wherein remaining activity was determined with 2.9 mM Ala-Pro-para-nitroanilide in 50 mM sodium phosphate pH 7.5; (c) activity towards Xaa-Pro-para-nitroanilide or Xaa-Ala-para-nitroanilide at ambient temperature in 50 mM sodium phosphate pH 7.5, wherein Xaa is selected from the group consisting of Ala, Arg, Asp, Gly, and Val; and (d) a molecular weight of about 93-96 kDa by
20 SDS-PAGE.

19. A protein hydrolysate produced by the method of claim 18.

20. A food product comprising the protein hydrolysate of claim 19.